Sustained Viral Suppression and Immune Recovery in HIV Type 1–Infected Children after 4 Years of Highly Active Antiretroviral Therapy

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We report the data from a long-term study of 31 human immunodeficiency virus type 1 (HIV-1)–infected children who were treated with highly active antiretroviral therapy. A high proportion of the children had undetectable HIV-1 RNA levels. $CD4^+$ T cell counts recovered and remained stable. Adverse events were observed frequently but were mostly mild.

Prospective studies on the use of HAART in HIV-1–infected children show viral suppression and recovery of the immune system. However, the response rates in children are highly variable and are frequently inferior to those observed in adults [1]. Most studies of HIV-1–infected children do not have an observation period that exceeds 48 weeks. Therefore, little is known about the durability of the viral suppression and the reconstitution of the immune system [1]. Here, we report the results at week 192 week (year 4) of a prospective, open-label cohort study on the clinical, immunological, and virological response to HAART in 31 HIV-1–infected children.

Methods. Protease inhibitor (PI)–naive HIV-1–infected children with a viral load >5000 copies/mL (measured on 2 consecutive occasions) and/or a $CD4^+$ cell count that was lower than their age-specific reference value were treated with indinavir or nelfinavir and 2 nucleoside reverse-transcriptase inhibitors. In all patients, steady-state intensive plasma pharmacokinetic sampling for the specific PI was performed. Pharmacokinetic sampling was repeated until the area under the plasma concentration-time curve reached target values [2]. Selected clinical data and laboratory values were obtained during regular visits to the outpatient department. Adherence to the treatment regimen was assessed by interviews with parents and patients, with medication diaries, and by measurements of plasma drug levels. All patients who started treatment before 1 January 2000 were included in the present study. The study protocol was approved by the medical ethics committee of the Erasmus Medical Center (Rotterdam, The Netherlands). Written, informed consent was obtained from patients and their parents.

At each time point, the percentage of patients with HIV-1 RNA levels that were less than the detection limit was calculated. For missing data, the response value was considered to be greater than the detection limit. Because absolute $CD4^+$ T cell and $CD8^+$ T cell counts are age related, $CD4^+$ and $CD8^+$ T cells counts as percentages of age-specific reference values were calculated. The patient's individual values at the different time points were divided by the median of the age-specific reference values for T cell subpopulations at that time point [3–5]. To evaluate growth, standard deviation scores (SDS) were obtained from the Dutch reference curves.

Differences between paired variables were analyzed with the Wilcoxon signed rank test and between groups with the Mann-Whitney *U* test. The relationship between response to therapy and baseline HIV-1 RNA level and age was evaluated using exact binary logistic regression analysis. For statistical analysis, SPSS, version 10 (SPSS); LogXact, version 4.1 (Cytel Software); Excel 97 (Microsoft); and Growth analyzer software were used.

Results. During the period of January 1997 through January 2004, a total of 31 HIV-1–infected children started HAART in our cohort. Their baseline characteristics are summarized in table 1. None of the children had received prior treatment with PIs or nonnucleoside reverse-transcriptase inhibitors.

At baseline, 28 children started HAART that included indinavir, and 3 children started HAART that included nelfinavir. Therapy was changed a total of 38 times for 28 children. Reasons for changing therapy included treatment failure ($n = 20$), drug toxicity ($n = 7$), simplification of the regimen ($n = 7$), refusal and/or intolerance of the medication regimen ($n = 3$), and failure to achieve appropriate pharmacokinetic values ($n = 1$). During follow-up, patients used a median of 2 different HAART regimens (range, 1–5 regimens). In 13 children (41% of all patients), HAART was changed at least once because of viral failure. Six patients were lost to follow-up. Reasons for study termination included death ($n = 1$), emigration

Received 8 July 2004; accepted 14 October 2004; electronically published 25 January 2005.

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Clinical Infectious Diseases 2005; 40:604–8

1058-4838/2005/4004-0018$15.00

Table 1. Baseline characteristics of the 31 HIV-1–infected children in a study of sustained viral suppression and immune recovery after 4 years of HAART.

Characteristic	Value
No. of subjects, male/female	16/15
Race	
White	4
Nonwhite	27
Age, median years (range)	5.1 (0.2–16.4)
Route of HIV transmission	
Vertical	22
Blood products	4
Unknown	5
CDC HIV disease classification[a]	
N1	2
N2	3
N3	1
A1	3
A2	5
A3	1
B1	2
B2	5
B3	1
C1	0
C2	2
C3	6
Prior NRTI treatment	
None	15
Any	16
AZT	11
AZT and zalcitabine	2
AZT and 3TC	1
AZT and ddI	1
AZT, ddI, and 3TC	1
Initial HAART regimen	
Idv, AZT, and 3TC	27
Idv, 3TC, and ddI	1
Nfv, AZT, and 3TC	1
Nfv, d4T, and ddI	2
Plasma HIV-1 RNA level, median copies/mL (range)	87,350 (725–2,195,000)
$CD4^+$ T cell value, median (range)	
Absolute cell count, $\times 10^6$ cells/mL	480 (0–3580)
Percentage of normal	47 (0–143)
$CD8^+$ T cell value, median (range)	
Absolute cell count, $\times 10^6$ cells/mL	1240 (180–5436)
Percentage of normal	155 (27.5–745)

NOTE. Data are no. of patients, unless otherwise indicated. AZT, zidovudine; CDC, Centers for Disease Control and Prevention; ddI, didanosine; d4T, stavudine; Idv, indinavir; Nfv, nelfinavir; 3TC, lamivudine.

[a] Clinical and immunological categories, as defined by the CDC.

($n = 2$), failure to come to the appointments ($n = 2$), and age of >18 years ($n = 1$).

One child died of serious invasive opportunistic infections 1 year after start of therapy. Three children had cases that showed progression in the Centers for Disease Control and Prevention (CDC) classification stage; none of these children had a serious pathology. The other children in the cohort were in good health, and a significant increase in growth was observed after the start of HAART (median body mass index ΔSDS, 0.44 [$P = .019$]; median height ΔSDS, 0.32 [$P = .05$]).

Figure 1 shows the proportion of patients with HIV-1 RNA levels that were less than the detection limits over time, assessed using as-treated and intention-to-treat (missing-equals-failure) analyses. After 4 years, 80% and 76% of the children had HIV-1 levels of <500 and <50 copies/mL, respectively, in the as-treated analysis, and 65% and 61% of the children, respectively, had such levels in the intention-to-treat analysis. Of the 25 children who were receiving treatment after 4 years, 7 (28%) had HIV-1 RNA levels that reached <500 copies/mL at week 12 and maintained HIV-1 RNA levels of <500 copies/mL during the entire follow-up period. These 7 patients were all considered to have been adherent to the treatment regimen, whereas 11 of the 18 patients who did not have complete viral suppression were considered to have been nonadherent to therapy at least once.

Binary logistic regression of the parameters of age, baseline HIV-1 RNA level, and viral response revealed no relation between the baseline HIV RNA level and therapy response ($P = .222$; $\beta = -.58$), but there was a significant negative relation between age and viral response rate ($P = .04$; $\beta = -.62$).

In figure 2*A* and 2*C*, the median $CD4^+$ T cell count relative to the normal value and the absolute $CD4^+$ T cells counts (with interquartile ranges [IQRs]) are depicted over time. Both the median value relative to the normal value and the absolute T cell count were significantly higher at week 192 than at baseline

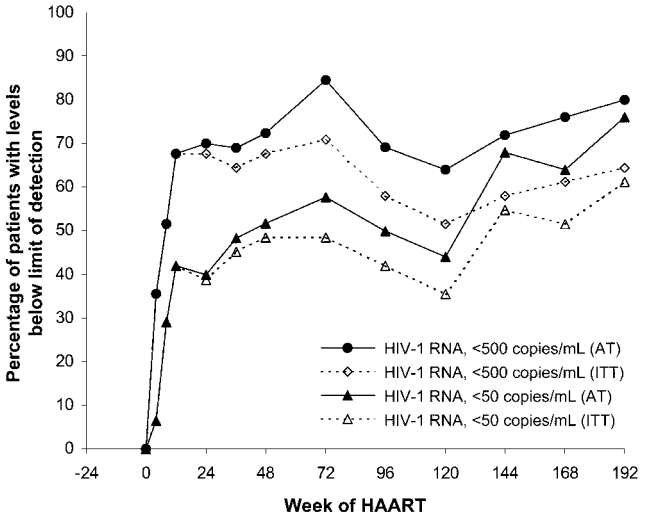

Figure 1. The proportion of children whose HIV-1 RNA levels decreased to <500 copies/mL and <50 copies/mL in as-treated (AT) and an intention-to-treat (ITT; missing-equals-failure) analyses.

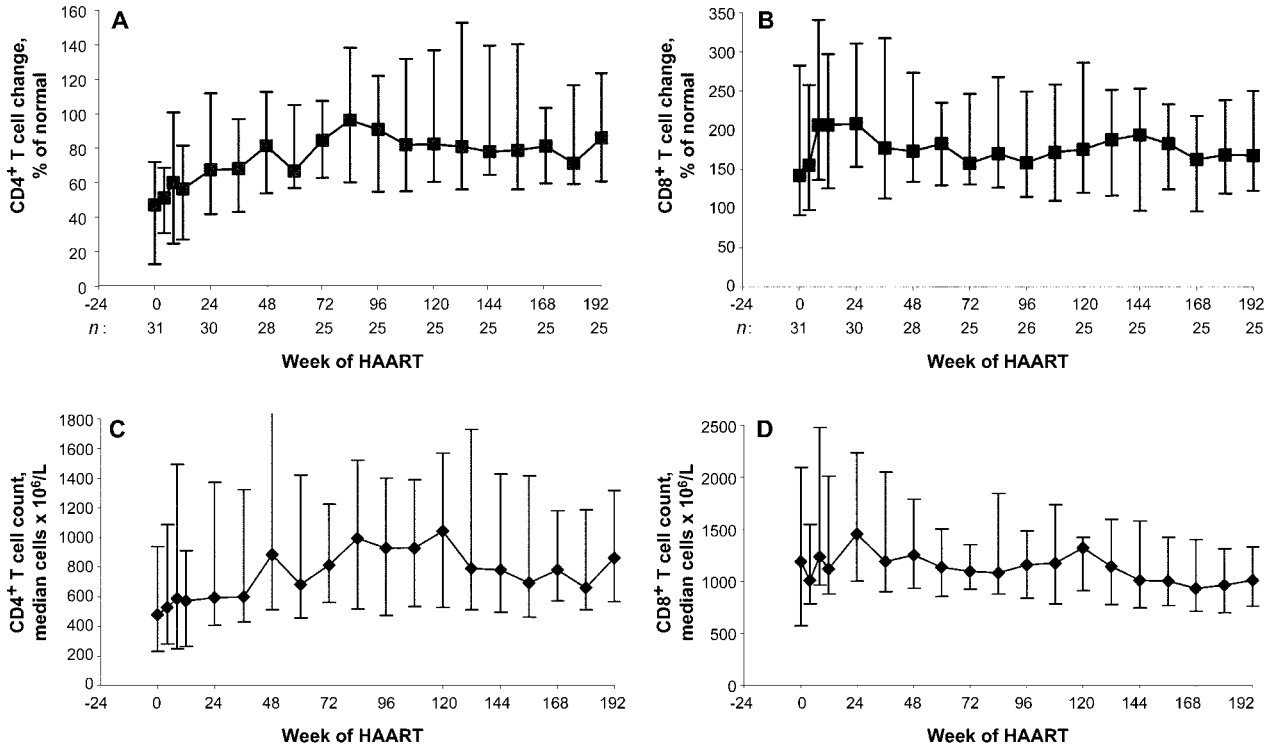

Figure 2. Median relative (i.e., percentage of normal) $CD4^+$ *(A)* and $CD8^+$ *(B)* T cell counts and median absolute $CD4^+$ *(C)* and $CD8^+$ *(D)* T cell counts for all patients.

($P = .01$ for the relative $CD4^+$ T cell count and $P = .025$ for the absolute $CD4^+$ T cell count).

In figure 2*B* and 2*D*, the median $CD8^+$ T cell count relative to the normal value and the absolute $CD8^+$ T cell count (with IQR) are depicted. Both remained high throughout the follow-up period and did not change significantly between baseline and week 192 ($P = .96$ and $P = .216$ respectively).

T cell function was analyzed by means of proliferative response to CD3 monoclonal antibody plus CD28 monoclonal antibody in whole-blood lymphocyte culture [3, 6]. The level of ^{3}H-thymidine incorporation increased after stimulation, from of a median of 345 counts/min/10^3 T cells (IQR, 134–865 counts/min/10^3 T cells) at baseline to 1186 counts/min/10^3 T cells (IQR, 640–1528 counts/min/10^3 T cells) at week 192 ($P = .07$). When expressed as percentages of the median, of 3450 adult healthy donors, the T cell response increased from a median of 17% (IQR, 7%–43%) at baseline to 59% (IQR, 32%–76%) at week 192 ($P = .07$).

Reconstitution of $CD4^+$ T cells (absolute $CD4^+$ T cell count and $CD4^+$ T cell count as percentage of normal) was not significantly different for virological responders and virological nonresponders. Likewise, the absolute $CD8^+$ T cell count did not significantly differ between responders and nonresponders. However, when normalized for age, starting at week 96, the median relative $CD8^+$ T cell count was lower in virological responders than in virological nonresponders (figure 3). The median $CD8^+$ T cell percentage at week 192 was 107% (IQR, 65%–126%) in responders and 205% (IQR, 161%–255%) in nonresponders ($P = .017$).

During follow-up, 24 patients (77%) reported having clinical adverse events. These were mostly mild and of gastrointestinal origin. In 7 children, the medication regimen was changed because of toxicity. In all cases, toxicity was associated with use of indinavir. For 1 patient, medication was changed because of skin rash; for all other patients, it was changed because of nephrotoxicity, including hematuria and flank pain ($n = 4$) and silent nephrolithiasis found on ultrasound examination ($n = 2$) [7]. Grade 3 or 4 laboratory adverse events were observed and included thrombocytopenia ($n = 3$), increased amylase levels ($n = 2$), and increased gamma-glutamyl transpeptidase levels ($n = 1$). None of these resulted in a change of therapy.

In 2 patients, lipoatrophy was suspected on the basis of anthropometric measurements. Both of the children in these cases used stavudine but different PIs (nelfinavir or indinavir). At the 4-year time point, fasting triglyceride and cholesterol levels could be obtained for 17 of the 25 children who were still receiving treatment. Both levels were not markedly increased,

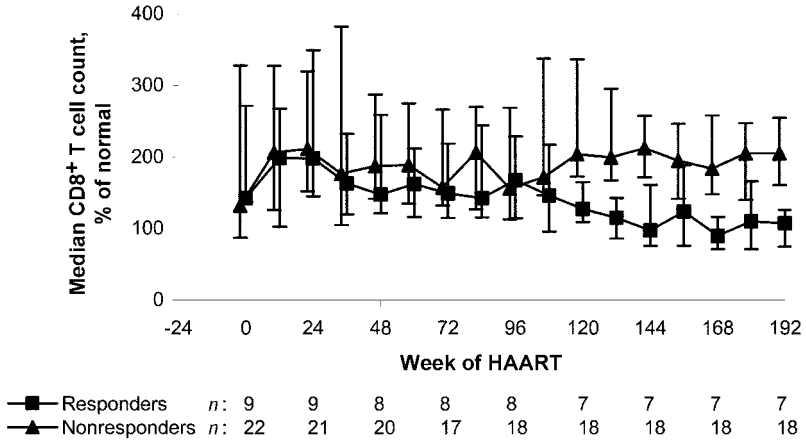

Figure 3. Change from baseline $CD8^+$ T cell count as percentage of normal in virological responders and nonresponders

with only 1 and 4 patients having cholesterol or triglyceride levels that were greater than the upper limit of normal, respectively.

Discussion. In the present study, a high proportion of children had a suppressed viral load after 4 years of treatment. Despite these good results, viral failure occurred often and frequently required changes in therapy. Strikingly, the proportion of children with a suppressed viral load increased during follow-up. We feel that this may be because of therapy changes and more-intensive intervention when nonadherence was suspected.

In contrast to other studies of children with an age at baseline similar to that of our cohort, a negative relation between age and viral response was found [8, 9]. We speculate that puberty-related problems that interfere with the adherence to HAART were more likely to occur because of the longer follow-up period in the present study.

After an initial increase, the $CD4^+$ T cell counts remained stable throughout the follow-up period. This indicated a durable effect of HAART use on the $CD4^+$ T cell population. The median $CD8^+$ T cell count and relative age-specific reference count remained high throughout the entire follow-up period, which indicated ongoing immune stimulation. This is different from data obtained in studies of adults in which $CD8^+$ T cell counts returned to baseline levels or even decreased to less than baseline levels after the initiation of HAART [10, 11]. Interestingly, a difference in $CD8^+$ T cell numbers as percentages of age-related reference values was observed for patients who had viral suppression throughout the study period and those who had not. This difference may be the result of decreased antigenic stimulation. However, the exact reason remains unclear and should be subject to further study.

In conclusion, an excellent response to HAART was observed. A high proportion of the children had undetectable HIV-1 RNA levels. $CD4^+$ T cell counts and T cell function recovered and remained stable throughout the follow-up period. Adverse events occurred frequently but were mostly mild.

Acknowledgments

We thank all of the children and their families, for participating in this study, and Katja Wolthers and Marijke Roos, for helpful discussion.

Financial support. All authors participated in studies financially supported by Merck Sharp & Dome, GlaxoSmithKline, and the Dutch AIDs Foundation from 1997 to 1999. From 2002 through 2003, the studies received financial support from Merck Sharp & Dome, GlaxoSmithKline, and Abbott.

Potential conflicts of interest. All authors: no conflicts.

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